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Patterns of co-occurring and mutually-exclusive mutations reveal synergistic interactions between cancer driver genes. A new study functionally confirms these interactions and builds the pairwise relationships into networks of pathway disruption that have better predictive power than relying on specific mutations.

Cancers are clonally expanding populations of cells in which the normal regulation of cell division and behaviour is undermined. That dysregulation is inherited by daughter cells and is thought to be facilitated by a small number of driver mutations. Genome sequencing studies comparing samples of cancer and normal cells from the same patient have been hugely successful at identifying the most commonly occurring driver mutations[1]. While it appears that most cancers have between one and ten principal drivers[2], it is also apparent that these mutations don't simply work in an additive manner to transform normal cells into cancer. Writing in this issue, Mina et al[3] set out to develop the idea that some driver mutations cooperate and so tend to co-occur in cancers, whereas others would be functionally redundant and tend to occur mutually-exclusively.

Studying the patterns of mutation co-occurrence and mutual-exclusivity are collectively referred to as evolutionary dependency (ED) analysis[4–8]. This new work substantially develops the concept beyond just pairwise dependencies, it systematically tests both assumptions of and predictions from ED, and gives a taste of the future insights that may be obtained from this style of analysis.

Functionally validated dependencies

Standing on the shoulders of The Cancer Genome Atlas (TCGA)[1], Mina et al[3] curated a list of recurrently mutated genes and their putative driver mutations. With a broad brush these were annotated as either oncogenes that drive cancer by dysregulated activity or gain of function, or tumour suppressor genes, where loss of activity enables cancer growth.

In an important validation of these driver annotations and proof-of-principal for later analyses, the authors turned to publicly available data from high-throughput gene essentiality screens of cancer derived cell-lines. These screens measure the relative fitness of cells - based on their ability to divide - after using short hairpin RNAs or CRISPR editing to deplete the products of a target gene[9–12]. Knowing the driver mutations already present within each cell-line, the authors asked if, as expected, depleting an oncogene with an activating driver mutation reduces cell fitness more than depleting the same gene in a cell-line that does not have the driver mutation. This worked remarkably well, confirming that the oncogene driver mutations were an important component of cell-line fitness, and to a lesser extent an equivalent strategy also validated tumour suppressor genes.

Having functionally validated many of the driver mutations they scored all pairwise combinations of drivers for co-occurrence and mutual-exclusion in the TCGA samples, finding a compelling excess of both patterns - suggesting a wealth of ED relationships.

A key insight was that the gene essentiality screens could be used to test these evolutionary dependencies and probe the nature of their interactions. For a pair of genes with an ED relationship, they identified cell-lines that contained driver mutations in both genes (double mutants), either gene (single mutants) or neither gene (wild-types), and asked how they responded to the depletion of one of the genes in the pair. Depleting a gene from a pair with co-occurring mutations tended to produce a much greater reduction in fitness for the double mutant cell-lines than for the single mutants (Fig. 1), supporting the notion of synergy between co-occurring driver mutations.

For pairs of driver mutations that tend to occur mutually-exclusively in cancers, depleting the mutated gene in single mutant cell-lines was found to be highly detrimental, more so than in double-mutant cell-lines. This supports the expectation that mutually-exclusive mutations are often redundant perturbations of the same cellular pathway.

Genetics has taught us to treasure our exceptions[13]. For the functional validation of co-occurring drivers there was a single prominent outlier, whereas ED predicted synergy between KRAS and STK11, the essentiality assays consistently point in the opposite direction, suggesting that STK11 mutations reduce a cell's need for KRAS driver mutations. Amongst mutually-exclusive driver pairs the exceptions were common, approximately 30% of significant essentiality assays indicating driver synergy, rather than ED implied redundancy. Reconciling these exceptions may provide greater insights than the confirmatory results, as they likely reflect the influence of the tumour microenvironment on driver gene interactions and could point to targetable cancer vulnerabilities.

Pieces of larger puzzles

Pairwise evolutionary dependencies are pieces of a larger puzzle. Mina et al[3], used ED to build "axes", networks of co-dependency and mutual-exclusivity, the aim being to classify cancers by the combinations of pathway perturbations that recurrently drive cancer development. Classification by axis appears to be better at predicting prognosis and drug response than stratifying on a single key driver mutation. Some of the drug responses in particular, such as the PARP-inhibitor sensitivity of PIK3CA/NFE2L2 double-mutants, would not have been predicted nor readily detected without axis based stratification.

The ED and functional validation approaches could be applied to other related puzzles such as whether distinct driver mutations in the same gene are functionally equivalent and contribute to the same axis. As cancer driver mutations appear to be common in non-cancer clonal expansions of cells that are a typical feature of aging[14,15], a comparison of ED axes between cancers and non-cancer clones may capture the distinctions between benign clonal expansion and cancerous transformation. Evolutionary inspired insights have a bright future lighting the paths of cancer development.

Fig. 1 | Paths of cancer development revealed by evolutionary dependency. a Patterns of significantly co-occurring or mutually-exclusive driver mutations (filled) identified for pairs of genes from cancer cohorts. **b** Gene product depletion in cell-lines often confirms synergy between co-occurring driver mutations and redundancy for mutually-exclusive mutations. Only one gene of the ED pair is depleted (red border) and the relative fitness compared between cell-lines stratified by the combined driver status (driver=filled, wild-type=open) of each gene. **c** Pairwise relationships can be

built into networks, within which clusters of co-occurring genes define an axis of cancer development. **d** Classifying cancers by axis can have greater predictive power than using single driver genes.

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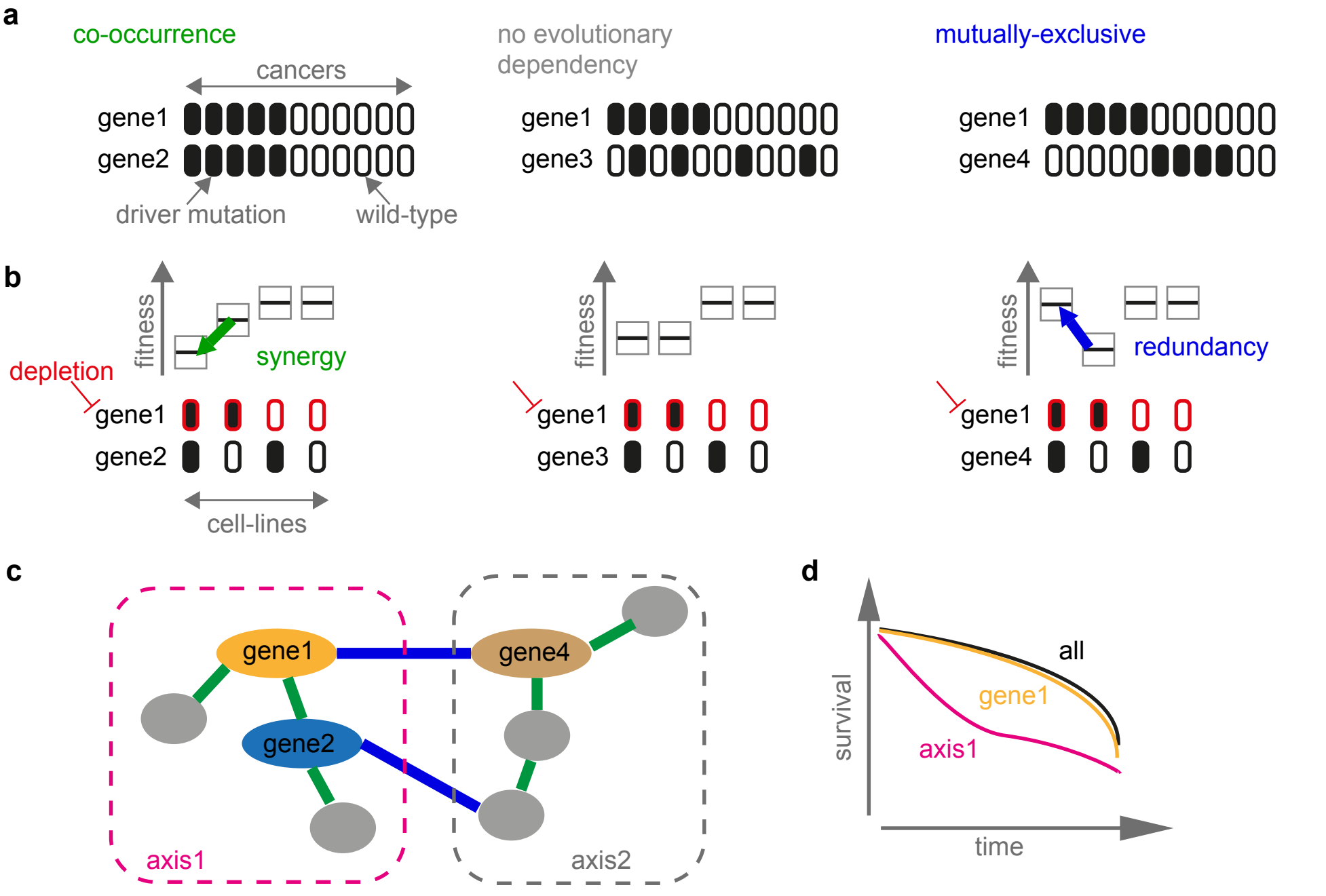
a
co-occurrence
cancers
gene1
gene2
driver mutation
wild-type
no evolutionary dependency
gene1
gene3
mutually-exclusive
gene1
gene4
b
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synergy
depletion
gene1
gene2
cell-lines
fitness
gene1
gene3
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gene1
axis1
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